REMARKS

Applicants have carefully studied the Final Office Action mailed on May 7, 2003 and Advisory action mailed on September 23, 2003, which issued in connection with the above-identified application. The present amendments and remarks are intended to be fully responsive to all points of rejection raised by the Examiner and are believed to place the claims in condition for allowance. Favorable reconsideration and allowance of the present claims are respectfully requested.

Applicants gratefully acknowledge the courtesy shown by the Examiner in providing recommendations for amendments and response to the present Office Action in a telephone interview with the undersigned and Dr. Paul Fehlner on November 5, 2003.

Pending Claims

Claims 2-30 were pending and at issue in the application. Claims 19-30 have been withdrawn from examination as being drawn to a non-elected invention. Claims 2-18 have been rejected under 35 U.S.C. § 103(a) as being obvious over the prior art.

In the present response, claims 21-30 have been canceled as drawn to a non-elected invention. Claims 19-20 have been withdrawn from consideration but not canceled, because, as discussed during the interview, these claims should properly be joined with claims 17 and 18. Claims 19 and 20 are product claims that depend from pending claim 18, which in turn depends from pending claim 17, both of which are product claims directed to a population of stem cells transduced with vector particles of the invention. Applicants respectfully submit that, in contrast to the Examiner's assertion, claims 19 and 20 do not recite any additional "steps" for generation of the cells of claims 17 and 18 but merely recite additional features of such cells. The recitation of % of cells expressing a gene of interest upon engraftment into a host simply constitutes a functional characteristic of the claimed stem cells, which can be revealed/tested upon engraftment. The presence of functional characteristics of this kind, along with structural

characteristics, is very common for patent claims in the field of biotechnology. In other words, claims 19 and 20 are typical dependent product claims and do not contain any method steps. If claims 17 and 18 are patentable, claims 19 and 20 must be also patentable because all limitations of claims 17 and 18 are intrinsically present in these claims. *See, e.g.*, 37 C.F.R. § 1.141(b).

In the present response, claims 3 and 17 have been amended to more particularly point out and distinctly claim the invention. Specifically, as suggested by the Examiner in a telephone conference on November 5, 2003, the phrase "substantially free of producer cells and producer cell supernatant" has been replaced with the phrase "free of producer cells and producer cell supernatant to the extent that the amount of these differentiation-inducing factors is too low to cause the stem cells to differentiate, proliferate, or die." The newly introduced recitation incorporates into the claim the definition of the term "substantially free" provided at page 15, lines 19-22 of the present specification. No new subject matter has been added as a result of these amendments, no new search is required, and no new issues are raised. The amendment merely explicitly recites the definition of "substantially free" in the specification. Upon entry of the above-identified amendments, claims 2-20 will be pending.

As discussed during the interview, the present claims call for a method for transducing stem cells using retroviral vector particles and stem cells transduced with the vector particles, wherein said vector particles (i) are pseudotyped with feline endogenous virus RD114 envelope protein and (ii) are substantially free of factors that induce stem cell differentiation by being free of both producer cells and producer cell supernatant to the extent that the amount of these differentiation-inducing factors is too low to cause the stem cells to differentiate, proliferate, or die, and whereby the transduced stem cells are capable of expressing the gene of interest and repopulating cell lineages when transplanted into a host. As reflected in the claim language, the two key features of the novel stem cell transduction/repopulation technology of the present invention are:

(i) the use of vector particles pseudotyped with feline endogenous virus RD114 envelope protein and

(ii) the realization that cells producing retroviral vector particles (*i.e.*, producer cells) and even cell-free producer cell supernatant contain factors that, upon transduction, induce stem cell differentiation and are therefore harmful for achieving multi-lineage engraftment (*see, e.g.*, page 25, lines 15-17, page 35, line 17 - page 36, line 33 and page 40, lines 11-22 of the present specification).

The present invention provides transduction methods and transduced cell populations capable of much greater engraftment efficiency than has been seen before. In addition, as discussed during the interview and in detail below, none of the prior art references suggest that the presence of differentiation-inducing factors is a problem, much less the solution to the problem, particularly for RD114 pseudotyped viral vectors.

35 U.S.C. §103(a) Rejections

In the Final Office Action, claims 2-18 stand rejected under 35 U.S.C. § 103(a) as being obvious over Hennemann *et al.* (Exp. Hematol., 1999, 27: 817-825) in view of the previously cited articles by Onodera *et al.* (J. Virol., 1998, 72: 1769-1774), Porter *et al.* (Hum. Gene Ther., 1996, 7:913-919), Uchida *et al.* (Proc. Natl. Acad. Sci. USA, 1998, 95:11939-11944), and Rebel *et al.* (Blood, 1999, 93: 2217-2224). The Examiner contends that Hennemann teaches a method of transduction of a retroviral vector into $CD34^{+}CD38^{-}$ cord blood stem cells pre-stimulated with cytokines, wherein (i) prior to transduction, producer cell medium containing viral particles is passed through a 0.45μm filter, (ii) transduction is performed using a plate pre-coated with fibronectin[1], and (iii) the transduced stem cells are capable of multi-lineage engraftment in SCID

[1] In the Final Office Action, the Examiner states that the plate is coated with fibronectin CH-296 (page 4, line 1 of the Office Action). Applicants respectfully note that, in contrast to the Examiner's assertion, the Materials and

(continued...)

mice. The Examiner concludes that it would have been obvious to combine the teachings of Hennemann with the teachings of Onodera and Porter to develop a method of transducing hematopoietic stem cells with retroviral vector particles pseudotyped with RD114. The Examiner further states that it would have been obvious to combine the teachings of Hennemann, Onodera and Porter with Uchida to develop an analogous method for letiviral vectors or to combine these teachings with Rebel to develop a method, wherein vector particles are freed of producer cells and producer cell supernatant by ultracentrifugation.

The rejection is respectfully traversed. Applicants respectfully submit that, even if taken together, the cited references do not disclose or suggest the transduction methods and transduced cells recited in the present claims and fail to provide motivation to combine as well as a reasonable expectation of achieving the invention.

Specifically, the present claims call for a method for transducing stem cells using retroviral vector particles and stem cells transduced with the vector particles, wherein said vector particles (i) are pseudotyped with feline endogenous virus RD114 envelope protein and (ii) are substantially free of factors that induce stem cell differentiation by being free of both producer cells and producer cell supernatant to the extent that the amount of these differentiation-inducing factors is too low to cause the stem cells to differentiate, proliferate, or die, and whereby the transduced stem cells are capable of expressing the gene of interest and repopulating cell lineages when transplanted into a host. As reflected in the claim language, the two key features of the novel stem cell transduction/repopulation technology of the present invention are:

(i) the use of vector particles pseudotyped with feline endogenous virus RD114 envelope protein and

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Methods section of Hennemann article simply states that plates were coated with fibronectin (Sigma) (page 819, bottom of the left column).

(ii) the realization that cells producing retroviral vector particles (*i.e.*, producer cells) and even cell-free producer cell supernatant contain factors that, upon transduction, induce stem cell differentiation and are therefore harmful for achieving multi-lineage engraftment (*see, e.g.*, page 25, lines 15-17, page 35, line 17 - page 36, line 33 and page 40, lines 11-22 of the present specification). Accordingly, as recited in the present claims, to obtain a population of transduced stem cells which are capable of achieving multi-lineage engraftment and efficient heterologous gene expression, it is critical that the vector particles used for transduction are substantially free of factors that induce stem cell differentiation by being free of both producer cells and producer cell supernatant. The preparation of vector particles is substantially free of such differentiation-inducing factors to the extent that the amount of these factors is too low to cause the stem cells to differentiate, proliferate, or die. Such differentiation-inducing factors can be eliminated, for example, by concentration of virus-containing producer cell culture medium using ultracentrifugation or pre-adsorbsion onto a surface that promotes adherence of the viral particles (*e.g.*, retronectin-coated plates).

Applicants respectfully note that, in contrast to the present invention, none of the references cited by the Examiner recognize or suggest the existence of any such differentiation-inducing producer-cell-derived factors and the importance of their elimination prior to stem cell transduction (see detailed comments below). If the Examiner believes that any of the cited references disclose or suggest a vector particle isolation/stem cell transduction method which is inherently capable of eliminating such differentiation-inducing producer-cell-derived factors, the Examiner is respectfully requested to provide evidence that this is necessarily and inevitably the case, and furthermore to establish motivation to apply such vector particle isolation/stem cell

transduction method to vector particles pseudotyped with feline endogenous virus RD114 envelope protein as recited in the present claims.[2]

Detailed discussion of each of the cited references is provided below.

In contrast to the present invention and contrary to the Examiner's assertion provided at page 4 of the Final Office Action and at page 2 of the Advisory Action, Hennemann does not teach that the viral particles used for transduction are substantially free of producer cell supernatant. As disclosed at page 819 of the article (second paragraph from the bottom in the left column), transduction is performed using virus-containing medium obtained by filtering the medium from producer cells through a 0.45μm filter. This treatment is a standard laboratory procedure for eliminating producer cells. However, most macromolecules and other components of the producer cell supernatant (including viral particles and much smaller differentiation-inducing producer-cell-derived factors) pass through the filter. Indeed, if the filtration step did not permit passage of the viral particles, the transduction would not succeed. In other words, the culture fluid of Hennemann is <u>not</u> substantially free of producer cell supernatant, as required by the present claims. The teaching of Hennemann can be further distinguished from the present claims because Hennemann does not disclose or suggest that the stem cell transduction should be

[2] Applicants respectfully note that, as stated in MPEP 2112, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ 2d 1955, 1957 (Fed. Cir. 1993); *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. *Ex parte Levy*, 17 USPQ 2d 1461, 1464 (Bd. Pat. App. & Inter. 1990). The analysis of the case law on the subject of inherency demonstrates that the fact that the prior art product may possibly have the same features as the claimed invention will not substantiate a finding of inherency. Rather, inherency must flow as a necessary conclusion from the prior art, not simply a possible one. *In re Oelrich*, 666 F.2d 578, 581, 212 U.S.P.Q. 323, 326 (C.C.P.A. 1981). To establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however may not be established by probabilities or possibilities. *In re Robertson*, 169 F.3d 743, 745, 49 U.S.P.Q. 2d 1949, 1950-51 (Fed. Cir. 1999).

performed using vector particles which are freed of the factors that induce stem cell differentiation. The absence of such teaching in Hennemann is further supported by the fact that, even when transduction was performed using fibronectin-coated plates, the virus-containing producer cell medium was not removed prior to the addition of stem cells (*see* page 819, paragraph at the bottom of the left column)[3]. In contrast, the very essence of the method of the present invention is exemplified by the method of first pre-loading viral particles on an adherent support (*e.g.*, retronectin-coated plates) and then removing the remaining producer cell medium and replacing it with fresh medium containing target stem cells (*see, e.g.*, page 32, lines 9-13 of the specification).

The secondary references do not cure the deficiency of Hennemann. Applicants respectfully note that, in contrast to the present invention and as acknowledged by the Examiner at page 4 of the previous Office Action, Onodera teaches only transduction using viral particles which are free of producer cells but are not free of the producer cell supernatant (*see, e.g.,* section entitled "Transduction protocol" in the right column at page 1770). As further acknowledged by the Examiner, Porter teaches transduction by co-cultivation of target cells with producer cells, *i.e.*, transduction using viral particles which are neither free of the producer cells nor free of the producer cell supernatant. In fact, Porter teaches away from the present invention by disclosing that co-cultivation of target cells with producer cells is a superior method because it maximizes the efficiency of infection (*see, e.g.*, page 915, left column, ¶3 and right column, ¶2, and Table 3 at page 917). In sum, even if combined, which is improper, Hennemann, Onodera and Porter do not teach the invention encompassed by the present claims, because they do not teach that the viral particles used for transduction should be substantially free of factors

[3] The Examiner's assertion provided at page 4 of the Final Office Action erroneously states that "Hennemann et al. teach that ... the producer cells and supernatant are substantially removed." As pointed out above, the 0.45μm filter removes the cells from the supernatant (filtrate), but does not remove the supernatant.

that induce stem cell differentiation by being free of both producer cells and producer cell supernatant to the extent that the amount of the differentiation-inducing factors is too low to cause the stem cells to differentiate, proliferate, or die.

Also, as acknowledged by the Examiner at page 4 of the Final Office Action (lines 1-2), in contrast to the present invention, Hennemann does not disclose viral particles pseudotyped with RD114 and therefore provides no motivation to be combined with Onodera and Porter which disclose RD114 pseudotyped viral particles. Conversely, neither Onodera nor Porter provide any motivation to be combined with Hennemann, because, in contrast to the instant invention and Hennemann, these references do not disclose that, upon transduction with viral particles, stem cells can be efficiently engrafted into a host to repopulate cell lineages. In fact, as admitted by the Examiner at page 4 of the Final Office Action, Onodera does not even disclose but merely suggests that transduction of stem cells with RD114 pseudotyped viral particles can be achieved. In short, the rejection requires combining teachings from the references selected to fit the template of the present invention and artificially rearranged in a way that would appear to coincide with the invention, when the references themselves teach the contrary: either the presence of producer cell supernatant (Hennemann, Onodera) or producer cells (Porter). Thus, the references lack the requisite suggestion to combine their teachings to achieve the claimed invention. *In re Fine*, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). The rejection relies on improper hindsight construction. *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992).

In the Final Office Action, the Examiner contends that Uchida supplements the disclosure of Hennemann, Onodera and Porter by teaching lentiviral vector-mediated gene transfer into hematopoietic stem cells. Applicants respectfully disagree and note that Uchida does not teach the salient limitation, removal of factors that induce stem cell differentiation by removing both producer cells and producer cell supernatant to the extent that the amount of these differentiation-inducing factors is too low to cause the stem cells to differentiate, proliferate, or die; nor does this reference provide any motivation to be combined with the other references,

because it does not disclose or suggest the use of RD114 protein to pseudotype the lentiviral particles. In fact, Uchida teaches away from the use of RD114 pseudotyped particles by describing superior stem cell transduction properties of VSV-G pseudotyped lentiviral particles. When a reference leads away from the claimed invention, as this one clearly does, *prima facie* obviousness is absent. See *In re Bell*, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993).

The Examiner further contends that Rebel supplements the teachings of Hennemann, Onodera and Porter by disclosing the use of ultracentrifugation to remove producer cells and producer cell supernatant and attain high titers of viral particles during transduction. In response, applicants respectfully submit that Rebel does not provide any motivation to be combined with the other references, because it does not disclose or suggest the use of RD114 pseudotyped retroviral particles.

In the Advisory Action, the Examiner argues that the teaching of ultracentrifugation of VSV-G pseudotyped vectors in Rebel does not teach away from the present invention because it "do[es] not teach that RD114 cannot be concentrated by the method of ultracentrifugation" (emphasis added). In other words, the Examiner suggests that Rebel might have considered in 1999 the possibility of using ultracentrifugation to concentrate viruses that are pseudotyped with envelope proteins other than VSV-G, and in particular viruses that are pseudotyped with RD114 envelope protein.

Applicants respectfully submit that this assumption is incorrect and relies on impermissible hindsight reconstruction based on the teachings of the present invention. It has been long acknowledged in the art that, because of the instability of the retroviral envelope, the retroviral vector particles cannot be efficiently concentrated to higher titers by ultracentrifugation. In VSV-G pseudotyped vectors, the retroviral envelope protein is replaced by the rhabdoviral G protein of the vesicular stomatitis virus, which is much more stable during centrifugation. In fact, VSV-G pseudotyped vectors have been developed specifically to improve the stability of retroviral vector particles (*see, e.g.*, Burns *et al.*, Proc. Natl. Acad. Sci.

USA, 1993, 90: 8033-8037 and Yee *et al.*, Proc. Natl. Acad. Sci. USA, 1994, 91: 9564-9568; attached as Exhibits A and B, respectively). Indeed, as stated at page 9564 (left column) of Yee *et al.* article:

> Attempts to concentrate retroviral vectors by physical methods such as filtration or ultracentrifugation have generally resulted in massive loss of infectious virus, presumably due to instability of the retroviral envelope protein, which is essential for the interaction of virions with the cell-surface receptor and for their entry into the cell... We have recently developed a method for pseudotyping Mo-MLV-derived retroviral vectors with the G glycoprotein of VSV. We have shown that the VSV-G pseudotyped vector can be concentrated to high titers by ultracentrifugation.

Accordingly, at the time of Rebel reference, it was accepted in the field that only VSV-G pseudotyped particles can be efficiently concentrated using ultracentrifugation, while retroviral envelope protein pseudotyped vector particles (including RD114 pseudotyped particles) cannot. Indeed, Rebel also stresses the superior properties of VSV-G pseudotyped viral particles at page 2217, left column and page 2222, concluding.

The ability to successfully concentrate vector particles pseudotyped with a retroviral envelope protein using ultracentrifugation represents an important and unexpected advancement of the present invention over the prior art. In contrast to accepted notion in the field shared by Rebel and similar references that only VSV-G pseudotyped vectors can be successfully concentrated by ultracentrifugation, the present invention provides novel and successful demonstration of the ability to concentrate vector particles pseudotyped with a retroviral envelope protein (*i.e.*, feline endogenous virus RD114 envelope protein) using ultracentrifugation. Accordingly, the Examiner's assumption with respect to Rebel is incorrect.

In summary, none of the cited references provide a suggestion of the transduction method and transduced cells recited in the present claims, much less a reasonable expectation of success. The actual teachings of the references taken as a whole do not suggest the claimed invention, and

the rejection requires impermissible hindsight reconstruction of various unconnected bits and pieces of the references to sustain itself.

Applicants respectfully submit that the Examiner's rejection does not meet the legal criteria for obviousness. The Examiner must show some objective teaching from the art that would lead an individual to combine the references, *i.e.*, there must be motivation. Thus, in considering obviousness, the critical inquiry is whether something in the prior art as a whole suggests the desirability, and thus the obviousness, of making a combination. *In re Newell*, 891 F.2d 899, 901-02, 13 USPQ2d 1248, 1250 (Fed. Cir., 1992). In particular, the mere fact that the teaching of a reference may be modified in some way so as to achieve the claimed invention does not render the claimed invention obvious unless the prior art suggested the desirability of the modification (emphasis added). *In re Fritch*, 972 F.2d 1260, 23 USPQ2d 1780, 1783 (Fed. Cir., 1992) and see also *Ex parte Obukowicz*, 27 USPQ2d 1063 (Bd. Pat. App. & Intf., 1993). In other words, determination that the invention is obvious requires that (i) cited references teach the claimed invention as a whole, and (ii) both the suggestion of making the invention, and a reasonable expectation of success can be found in the prior art, not in the applicants' disclosure. MPEP Section 2143; *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir., 1988); *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir., 1991). The courts have held that it is improper to use hindsight to combine elements found in the prior art to arrive at a determination of obviousness. *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1532 (Fed. Cir. 1988); *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992); *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1552 (Fed. Cir. 1983). In the present case, the references provide neither the suggestion to modify transduction procedures with viral vectors by making them substantially free of factors that induce stem cell differentiation, much less provide any reasonable expectation of success in doing so.

In light of the foregoing legal considerations and arguments, it is respectfully submitted that pending claims are not obvious over the cited art. Reconsideration and withdrawal of the obviousness rejection is believed to be in order.

CONCLUSION

Applicants request entry of the foregoing amendments and remarks in the file history of this application. In view of the above amendments and remarks, it is respectfully submitted that claims 2-20 are now in condition for allowance and such action is earnestly solicited. If the Examiner believes that a telephone conversation would help advance the prosecution in this case, the Examiner is respectfully requested to call the undersigned agent at (212) 527-7634. The Examiner is hereby authorized to charge any additional fees associated with this response to our Deposit Account No. 04-0100.

Respectfully submitted,

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